Preeclampsia and the C282Y Mutation in the Hemochromatosis (*HFE*) Gene

To the Editor:

The hemochromatosis (*HFE*) gene encodes the HFE protein, a transmembrane glycoprotein that is implicated in the modulation of iron uptake from the diet *(1)*. The C282Y mutation in this gene is known to be associated with moderately increased serum iron indices. Recently, several studies have described an association between increased maternal iron status and an unfavorable pregnancy outcome *(2, 3)*. Preeclampsia is one of the most common pregnancy-related syndromes as well as a major cause of fetal and maternal morbidity and mortality. Preeclampsia is defined by de novo hypertension and proteinuria in pregnancy. The etiology of preeclampsia is complex and not fully understood, but abnormal placentation and endothelial dysfunction may play an important role in its pathogenesis. Abnormal placentation may lead to a malperfused placenta with release of toxic iron through hemoglobin or heme, which finally may contribute to generalized endothelial dysfunction *(2)*. In the present study, we analyzed the association between the C282Y allele and the presence of preeclampsia in a case–control study described previously *(4)*. The analysis was restricted to those patients and their controls who fulfilled recent criteria for preeclampsia (157 women in each group).

The *HFE* C282Y genotype was determined by an automated method using minor-groove-binding DNA oligonucleotides (MGB probes) as described previously *(5)*. The presence of a C282Y allele was confirmed by conventional PCR with restriction fragment length polymorphism analysis.

A Fisher exact test with a 0.050 one-sided significance level showed 90% power to detect the difference between a C282Y allele frequency of 10% (estimated population frequency) and a case frequency of 22.5% (odds ratio = 2.8) when the sample size in each group was 157.

The observed frequency for the *HFE* C282Y allele was similar for women with preeclampsia and controls (0.070). In addition, there was no significant difference between cases and controls in the frequency of the three genotypes [wild type (wt)/wt, wt/C282Y, and C282Y/C282Y] or the presence of the C282Y allele (wt/C282Y and C282Y/C282Y; Table 1). Furthermore, we found that adjustment for clinical indices previously positively (familial hypertension and body mass index) and negatively (smoking) associated with preeclampsia in a logistic model had no significant effect on the distribution of the various C282Y genotypes among women who developed preeclampsia and controls.

The finding of no differences in the C282Y allele distribution or the frequency of the C282Y allele between women with pregnancies complicated by preeclampsia and controls with uncomplicated pregnancies suggests that the C282Y polymorphism is not predominantly involved in the development of endothelial cell dysfunction as a result of increased iron caused by this mutation in women with preeclampsia.

In the total group of cases and controls we found a high frequency (1 of 63) of women who were homozygous for the C282Y allele, whereas 1 in 9 were heterozygous for this allele. The proportion of pregnant women in the present study who had the C282Y/C282Y genotype (1.6%), however, did not differ significantly from the frequency of 0.3–0.5% reported previously for individuals of Northern European descent *(1)*.

In conclusion, our data do not support the hypothesis that the C282Y allele of the hemochromatosis (*HFE*) gene is a clinically important marker of an increased risk for the development of preeclampsia.

Table 1. Genotype frequencies in women with preeclampsia and control women.

Genotype	Women with preeclampsia (n = 157)		Controls (n = 157)		Odds ratio[a] (95% confidence interval)
	n	%	n	%	
wt/wt	137	87.2	138	87.9	1.0
wt/C282Y	18	11.5	16	10.2	1.13 (0.56–4.27)
C282Y/C282Y	2	1.3	3	1.9	0.67 (0.11–3.12)
wt/C282Y or C282Y/C282Y	20	12.8	19	12.1	1.06 (0.54–4.08)
fC282Y[b]		0.067		0.070	

[a] Odds ratios were determined by multiple logistic regression analysis.

[b] Frequency of the C282Y allele. For women with preeclampsia and controls, the observed vs expected allele frequencies are 1.27% (95% confidence interval, 0.15–4.5%) and 1.91% (0.4–5.5%), respectively.

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Influence of Preanalytical Factors on the Immulite Intact Parathyroid Hormone Assay

To the Editor:

Preanalytical factors that affect parathyroid hormone (PTH) concentrations are not well defined. Measured PTH concentrations in EDTA plasma may *(1, 2)* or may not *(3)* differ from those in serum. PTH has been reported variously to be stable for hours to days *(1–6)*. In addition, different PTH assays cross-react to various extents with biologically inactive, N-terminally truncated PTH molecules *(7)* that may have different stabilities. The specificity of a given immunometric assay depends on the binding sites of the anti-PTH antibodies used. So-called "intact" PTH (iPTH) assays show cross-reactivity with peptides lacking N-terminal amino acids. Assays measuring only PTH with intact NH_2 termini are named CAP™; whole PTH, 3rd generation; or bioactive PTH assays *(7)*.

In 2001, DPC introduced a reformulated iPTH sandwich assay (starting with lot no. 106) in which the polyclonal tracer antibody had been replaced by a monoclonal antibody raised against an epitope within the first 34 amino acids of PTH. With this new method, reference intervals and, in particular, PTH concentrations measured in serum samples of dialysis patients are lower (data not shown). In March 2003, DPC reported underrecovery of PTH with this method *(8)*, and a new lot of raw materials was introduced, starting with assay lot no. 112. The calibration for the Immulite analyzer was adjusted at assay lot no. 113 *(8)*. Shortly before this, we had found that PTH concentrations in EDTA plasma measured with lot no. 109 apparently increased with time (data not shown), whereas a previous report *(1)* indicated that PTH as measured by this method was stable in EDTA plasma *(1)*. The changes in the PTH assay and the conflicting results on PTH stability in EDTA samples led us to investigate with the newer monoclonal/polyclonal iPTH assay both PTH reference intervals and stability as well as agreement between the PTH measurements on the Immulite and Immulite 2000 analyzers.

Reference intervals were estimated by the nonparametric method in Analyze-It for Microsoft Excel, based on results for material from nonpregnant volunteers from the outpatient clinic (age range, 20–65 years). Individuals suffering from renal failure or taking vitamin and/or mineral supplements were not included in the study. All samples were obtained between 0900 and 1300. Almost all volunteers (98%) were Caucasians; 61% were female, and 39% were male. Blood was collected into two dipotassium EDTA Vacutainer™ tubes (plastic; BD) and into a serum tube with clot activator and SSTII™ gel. Heparin plasma was not included in the study because measurement of PTH in heparin plasma frequently led to inexplicable outliers (data not shown). After venipuncture, one EDTA tube was immediately put into melting ice (EDTA*ice*), the other EDTA and the serum tube were kept at room temperature. Serum tubes were allowed to clot before centrifugation. EDTA*ice* plasma was centrifuged at 4 °C. PTH was assayed on an Immulite 2000 (assay lot no. 112) and an Immulite (assay lot no. 113) analyzer within 2.5 h of venipuncture, according to the manufacturer's protocols.

Of the 137 volunteers, 9 had PTH concentrations more than 1.5 interquartile ranges from the first quartile above the median and were investigated as possible outliers. All nine samples had pathologically low calcium concentrations or increased plasma phosphate or creatinine concentrations and thus were excluded from the reference interval study. To assess the stability of PTH, we retested samples that had been stored as serum and plasma at 4 °C on the Immulite 2000 48 h after the initial measurements.

PTH measured in serum was lower than in EDTA or EDTA*ice* plasma ($P < 0.0001$, Wilcoxon signed-ranks test). For comparison Table 1 also contains results from a similar reference interval study we had conducted previously with lot no. 109. The PTH concentrations found with lot no. 112 on the Immulite 2000 were slightly higher than in the reference interval study with lot no. 109. When we used the same samples, the reference intervals determined with the Immulite were higher than those determined with the Immulite 2000.

Table 1. Median PTH concentrations and estimated nonparametric 95% reference intervals obtained on Immulite and Immulite 2000 analyzers.

Analyzer	PTH assay lot no.[a]	No. of samples	Material	Median, pmol/L	Reference interval, pmol/L
Immulite	113	124	Serum	3.5[b]	1.4–6.3
		124	EDTA	4.6	2.0–8.3
		124	EDTA*ice*	3.9	1.5–7.8
Immulite 2000	112	128	Serum	2.8[b]	1.2–5.2
		128	EDTA	3.8	1.6–7.0
		128	EDTA*ice*	3.3	1.4–6.1
Immulite 2000	109	134	Serum	2.1[b]	0.7–4.9
		121	EDTA	2.7	0.9–5.8
		135	EDTA*ice*	2.4	0.6–5.2

[a] Lot nos. 112 and 113 are "improved" lots of the monoclonal/polyclonal assay reagent sets, whereas lot no. 109 is from the original monoclonal/polyclonal assay reagent set.

[b] $P < 0.0001$ vs corresponding EDTA or EDTA*ice* samples (Wilcoxon signed-ranks test).